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RAPID REPORT

Human Albumin Solder Supplemented With TGF-β_1 Accelerates Healing Following Laser Welded Wound Closure

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Background and Objective: **We examined the possibility that human albumin solder can be used as a vehicle for site specific delivery of growth factors for the purpose of accelerating tissue repair following laser welded wound closure. Certain human recombinant growth factors have been shown to accelerate wound healing in model systems. Pilot in vitro studies have established that several growth factors, including TGF-β_1, maintain bioactivity following exposure to temperatures achieved during laser tissue welding. Using a temperature controlled laser delivery system (TCL) to precisely maintain welding temperatures, it is now possible to avoid thermal denaturation of exogenous bioactive molecules such as growth factors.**

Study Design/Materials and Methods: **HB-EGF, bFGF, and TGF-β_1 were tested in vitro for maintenance of bioactivity after exposure to 80°C. In vivo experiments using porcine skin determined the efficacy of solders augmented with growth factors. Incisions were repaired using human albumin alone or supplemented with HB-EGF (2 μg), bFGF (10 μg), or TGF-β_1 (1 μg). Wounds were excised at 3, 5, and 7 days post-operatively. Tensile strength, total collagen content, and histology were performed.**

Results: **At 3 days, tensile strength (TS) of TGF-β_1 wounds were 36% ($P < 0.05$) and 20% (n.s.) stronger than laser alone and suture closures, respectively. By 5 days the TS of the TGF-β_1 group increased by 50% ($P < 0.05$) and 59% ($P < 0.02$) over laser alone and suture groups, respectively. At 7 days the TGF-β_1 group was 50% ($P < 0.05$) and 79% ($P < 0.01$) stronger than laser solder alone or suture, respectively. The HB-EGF and bFGF groups were equivalent to the laser solder group at all time points. Total collagen**

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content at 7 days increased in the TGF-β_1 group by 7% (n.s.) over the suture group and 21% ($P < 0.05$) in the laser group.
Conclusion: **Human albumin solder supplemented with TGF-β_1 increases the early post-operative strength of laser welded wounds. This novel application of laser tissue soldering augmented with a growth factor has the potential to bring about immediate fluid tight seals while providing site specific delivery of biological modifiers. This may lead to an overall improvement in post-operative convalescence, wound infections, and hospital costs.**

INTRODUCTION

Current methods for surgical wound closure involve the approximation of tissue segments with sutures, clips, or staples. Although these techniques are generally reliable, there are a number of limitations that have prompted a search for alternative methods of wound closure. One promising approach is the use of laser assisted tissue repair (LATR) to photothermally fuse biological tissues. There are several advantages of LATR over conventional suturing methods. Laser welded wound closure is significantly faster and less traumatic than conventional suture repair. In addition, improved healing may result from a reduced inflammatory response by virtue of the absence of foreign bodies (suture) at the repair site. Further, laser assisted wound closure can provide higher acute leak point pressures when compared to wounds closed with sutures [1–6].

Yahr and Strully were first to report on the use of LATR to perform tissue approximation [7]. Initial attempts using laser assisted tissue fusion often ended in repairs with low tensile strength resulting in premature leak and rupture [8,9]. To overcome this difficulty, tissue solders were developed to improve the strength of the laser weld [1]. Of the tissue solders tested, human albumin solder has been documented to enhance LATR resulting in repairs with clinically acceptable tensile strength and integrity [4,5]. The albumin solder is denatured at the repair site during laser welding to enhance the adhesion of the adjacent tissue edges. The resulting denatured solder is non-immunogenic, and is gradually degraded in the normal wound healing process.

Although laboratory research has demonstrated improved wound repair with laser welding, the widespread clinical use of LATR has been limited. This hesitancy to move into the clinical environment stems mainly from the highly subjective visual endpoint used during laser tissue fusion. Applying visual end points to determine when a successful weld has occurred makes a reproducible laser closure difficult to achieve. One method for standardizing the end point for laser welding relies on controlling the tissue temperature [11]. A temperature controlled laser welding system is capable of maintaining a constant predetermined tissue temperature during welding. Controlling the tissue temperature should eliminate the subjective end point and provide a means for reproducible laser wound closure.

The concurrent development of albumin tissue solder to increase the acute LATR tensile strength and temperature control systems for more reliable closures has spawned the evolution of the novel tissue repair model presented in this paper. A number of reports have indicated that specific growth factors applied exogenously to wounds are bioactive and accelerate healing in model systems [12–14]. We reasoned that growth factors combined into an albumin tissue solder might facilitate healing. This is because, following LATR closure, these biologically active factors would be released locally from the denatured albumin solder and become available to appropriate target cells. Thus, the LATR system incorporating temperature control may provide a model for controlled delivery to study quantitatively the effects of growth factors on wound healing. This report describes the first attempt to test this concept in an in vivo system.

METHODOLOGY

Materials

Recombinant human bFGF was generously provided by Dr. Judith A. Abraham (Scios Nova Inc., Mountain View, CA). TGF-β_1 was provided by R&D Systems, Inc. Minneapolis, MN. HB-EGF (Catalog # 259-HE) was obtained from R&D Systems, Minneapolis, MN. Human albumin was kindly provided by Dr. Gerry Marx (The New York Blood Center/Melville Biologics, New York, NY). Human albumin tissue solder was prepared using a technique previously described by our laboratory [15]. For solders containing human re-

combinant growth factors, the growth factors were solubilized in a 4 mM HCl solution and added to 1 × PBS immediately prior to albumin reconstitution. All solders were prepared the day prior to use and allowed to mix overnight at 4°C to ensure even distribution of the growth factor throughout the solder.

Growth Factor Evaluation: Heat Stability

Based on recent data, temperatures between 65°C and 80°C appear to be optimal for LATR of skin [11]. Accordingly, growth factors were evaluated for heat stability at 80°C in a water bath (2 minute exposure) or using a temperature controlled photocoagulation system [11]. HB-EGF[16], bFGF[13], and TGF-β_1 [13,14] were selected for evaluation based on published results indicating they are involved in wound healing. All growth factors were tested in vitro using a concentration of 500 ng/ml. The following sample preparations were evaluated for bioassay: 1) Growth factor (GF) in 1 × PBS containing 0.1% BSA-not heated; 2) GF in 1 × PBS containing 0.1% BSA-heated; 3) GF in 50% human albumin-not heated; 4) GF in 50% human albumin-heated. The samples of albumin solder that were heated resulted in denaturation of the albumin vehicle forming a solid opaque mass. The denatured albumin was homogenized in an equal volume of 1 × PBS. The solution was centrifuged at 3,000 rpm for 5 minutes. The supernatant was evaluated for growth factor bioactivity (methods described below). For solder samples heated using the temperature control system, a 3 mm × 40 mm layer of solder (approximately 200 μm thick) was irradiated employing a set temperature of 80°C using a technique similar to that used during tissue welding. The laser denatured solder was homogenized as described above. All samples were maintained at 4°C until assayed. The bioactivity of HB-EGF and bFGF samples, were determined in a BALB/c 3T3 cell proliferation model previously described [17]. Bioactivity of the TGF-β_1 samples were determined using a plasminogen activator inhibitor luciferase reporter assay described previously [18].

LATR Surgical Model: Wound Healing

The protocol employed was approved by the Institutional Animal Care and Use Committee. All animals used in the study received humane care in compliance with the "Principles of Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, published by the National Institutes of Health (NIH Publication No. 80-23, revised 1985).

A porcine incisional skin model was chosen as it is considered to be a good model for studying wound repair, and it is morphologically similar to human skin [17,19]. Briefly, 2 cm full-thickness incisional wounds were created on the dorsum of male Yorkshire swine (12–15 kg) using a #11 scalpel blade (methods previously published) [11]. Wounds were irrigated with 1:100,000 epinephrine to minimize bleeding. Incisions were repaired using one of the repair techniques described below. Conventional wound closures were performed using two interrupted 5-0 Prolene sutures evenly spaced across the repair. All wounds for LATR were initially approximated using a single 5-0 Prolene suture placed in the center of the wound. Following laser repair, the suture was removed. LATR wound closures were performed using a 1.32 micron laser (Premier Laser Systems, Inc., Irvine, CA) connected to a remote temperature control system [11] (Abiomed R&D, Inc., Danvers, MA), which was set to weld at a constant temperature of 70°C (± 3°C) at a rate of 0.4 mm/second. When protein solder was used, a 50 μl bolus was applied between the wound edges immediately prior to LATR. The solder was allowed to completely cover the surface area of the approximated wound edges before laser irradiation.

Following repair in the survivor studies, a clear sterile Opsite dressing was applied. A total of 10 wound samples were generated for each repair group per time period. One sample from each group was evaluated for total collagen content. A second sample was retained for histologic analysis. The remaining eight samples were tested for tensile strength using an Instron Model Mini-44 tensiometer (Instron, Inc., Canton, MA). Tensile strength was measured as the maximum stress (in kiloPascals) to completely separate the repair edges. Total collagen concentration was measured in a hydroxyproline assay with the assumption that collagen contains 14% hydroxyproline (methods previously described) [20]. Histologic analysis was performed using hemotoxylin/eosin and Masson's trichrome stains.

Unpaired Student's *t*-tests were employed to determine significant differences between groups.

RESULTS

Three growth factors were chosen to evaluate the use of LATR with growth factor enhanced albumin solder: (1) transforming growth factor-beta

1 (TGF-β_1), a secretory product of platelets, known to be a multifunctional inducer of extracellular matrix, a mitogen for stromal cells, and an immunosuppressive factor [14], TGF-β_1 has been studied extensively as a important mediator of wound healing in several models [9,21]; (2) basic fibroblast growth factor (bFGF/FGF-2), a mitogen for cells of mesenchymal origin and a potent angiogenic factor [22,23]. Since bFGF has been shown to be thermally labile [24], its bioactivity may be compromised by the LATR process; and (3) heparin-binding epidermal growth factor-like growth factor (HB-EGF), an EGF receptor ligand and mitogen for fibroblasts and epithelial cells [16]. HB-EGF has been shown to be thermally stable, and is expressed transiently during healing in the porcine skin and has been implicated in the host response to tissue repair [17].

Growth Factor Enhanced Solders: Thermal Stability Studies

Pilot studies were completed to determine if a protein solder developed for LATR can be used as a carrier for growth factors after thermal denaturation of the solder. Of particular interest was the determination of whether the growth factors added to the protein solder remained bioactive following heating to the temperatures encountered during LATR. An in vitro model was chosen to allow a quantitative measurement of the bioactivity of the growth factors both before and after heating.

A 50% (w/v) solution of human albumin has been shown previously to perform as a functional tissue solder using a 1.32 μm Nd:YAG laser [3]. Preparations of this albumin solder containing bFGF, HB-EGF, and TGF-β_1 at 500 ng/ml concentration were tested using standard assays after heating in a water bath at 80°C for 2 minutes, or after heating to 80°C using LATR under temperature control. Bioactive bFGF, HB-EGF (Fig. 1), and TGF-β_1 (Fig. 2) were released from the thermally denatured albumin solder, when a dialyzed supernatant of the homogenate of these solders was analyzed. When compared to heated controls containing growth factors in PBS, no significant difference in growth factor activity in the denatured albumin's supernatant was observed with bFGF or HB-EGF. This suggests these growth factors are capable of diffusing from the denatured solder. Interestingly, when bFGF was heated in a PBS vehicle alone, approximately 50% of its bioactivity was lost. This is consistent with the known heat-sensitivity of bFGF; however, this result suggests that in the presence of high concentrations of albumin, bFGF bioactivity is preserved following exposure to heat.

Unlike bFGF and HB-EGF, which are fully recoverable from a PBS vehicle (Fig. 1), TGF-β_1 in PBS was only recoverable at 10–20% (Fig. 2).

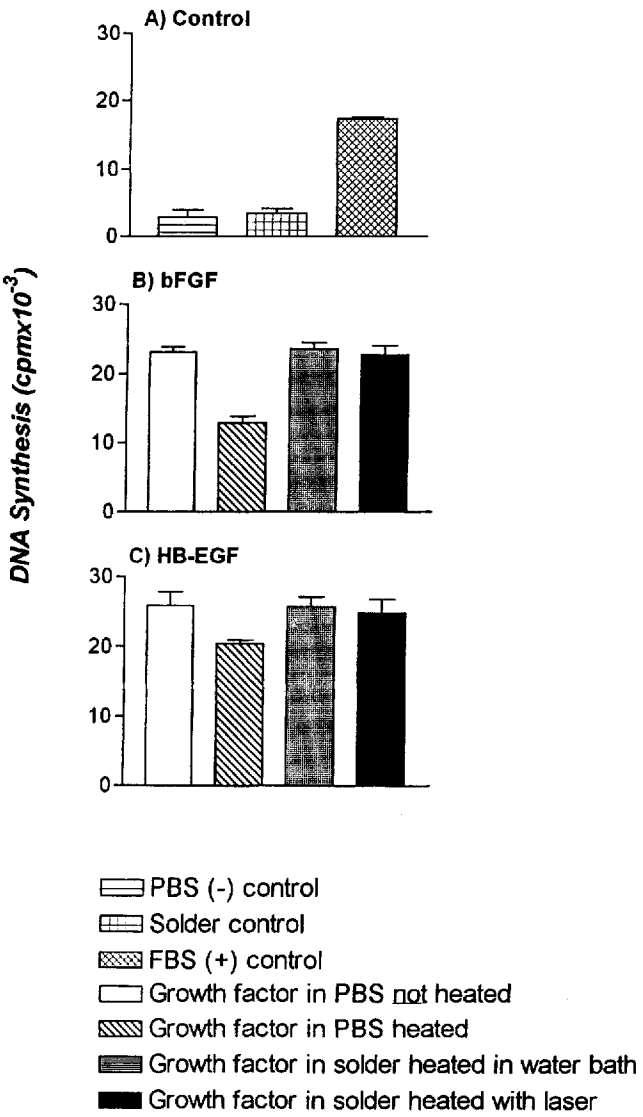

Fig. 1. Results of 3T3 mitogenic assay for (A) controls, (B) bFGF, and (C) HB-EGF. A marked decrease in bioactivity occurred following heating of both growth factors in PBS. (>50% reduction for bFGF). No decrease in bioactivity was seen following heating of either growth factor in 50% albumin. This indicates that albumin may protect the growth factors from thermal inactivation.

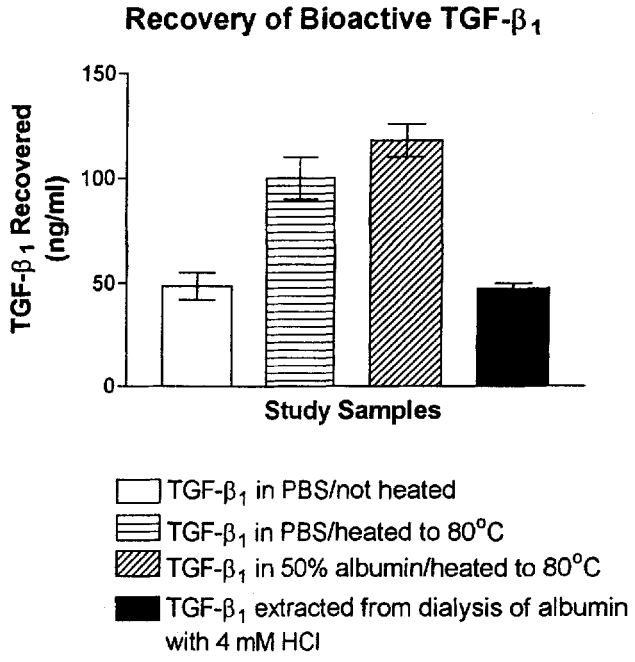

Fig. 2. Results of luciferase reporter assay for TGF-β_1 (500 ng/ml). A 10 to 20% recovery of biologically active TGF-β_1 was observed for all PBS and 50% albumin samples. This suggests TGF-β_1 remains bioactive following heating. TGF-β_1 has been shown to bind avidly to the plastic tube used in this assay [25]. This may account for the overall low recovery of the protein. When further dialysis of the albumin homogenate was performed with 4 mM HCl, additional bioactivity was recovered. This suggests that TGF-β_1 may be trapped or bound in the denatured albumin.

This low recovery occurs because TGF-β_1, as demonstrated by Brown et al., binds avidly to the plastic test tubes used in the assay [25]. The recovery of bioactive protein from heated 50% albumin was equivalent to that of PBS suggesting that TGF-β_1 is heat stable and remains bioactive. This finding is consistent with a previous report demonstrating that TGF-β_1 is thermally stable to temperatures under 90°C [25] (Fig. 2). However, when the remaining denatured albumin homogenate was further dialyzed with a weakly acidic solution (4 mM HCl), additional bioactivity was measured. This result suggests that TGF-β_1 may be trapped in the albumin solder during the thermal denaturation process, which contrasts with the results obtained for bFGF and HB-EGF, where virtually all of the growth factors were recovered by a single PBS dialysis. Furthermore, this in vitro data indicates that growth factors belonging to three gene families, with distinct structural and biochemical properties, can be released from a tissue solder in a bioactive form after thermal heating.

LATR Surgical Model: Wound Healing Studies

We next sought to characterize any biological or functional effect of albumin solders supplemented with three growth factors (albumin + growth factors) in experiments using LATR. Albumin solders containing HB-EGF, bFGF, and TGF-β_1 were applied to 2 cm full-thickness incisional wounds in porcine skin, which were closed using LATR techniques with constant temperature control (70°C). Measurements of tensile strength were performed acutely, and 3, 5, and 7 days post-operatively (Fig. 3). No differences were observed between the three albumin + growth factor solders and the albumin solder control immediately after the repair (acute group). In addition, no difference was measured in the wound tensile strength for either the albumin + HB-EGF or the albumin + bFGF solders, vs. the control solder for all post-operative measurements (at 3, 5, or 7 days). The albumin + TGF-β_1 solder, however, showed a significant enhancement of tensile strength at 5 and 7 days after the repair ($P < 0.05$).

A second series of experiments were completed to compare the healing rate of albumin + TGF-β_1 solder to a conventional suture closure. In these experiments, wounds were closed with LATR techniques using either a pure albumin solder, an albumin + TGF-β_1 solder or two evenly spaced sutures (suture control). Suture closures and laser welded closures with solder alone showed similar functional effects, as measured by increases in tensile strength vs. post-operative day (Fig. 4). Consistent with the previous data, LATR closures using the albumin + TGF-β_1 solder showed a significantly accelerated rate of healing when compared to the LATR closures completed with the control solder ($P < 0.05$) and the conventional suture closures ($P < 0.02$).

To verify the functional tensile strength data qualitatively, measurements of total collagen content at the repair site were made for each of the various surgical techniques described above. A significantly higher collagen content was measured for the albumin + TGF-β_1 group at 7 days ($P < 0.05$), compared to the other closure groups (Fig. 5). Histologic analysis also suggested a more abundant accumulation of newly formed collagen in the albumin + TGF-β_1 repair group (vs. the other experimental groups) at post-operative day 7 as evaluated by Masson's trichrome stain (Fig. 6). These data support the functional tensile strength measurements, and further suggest that

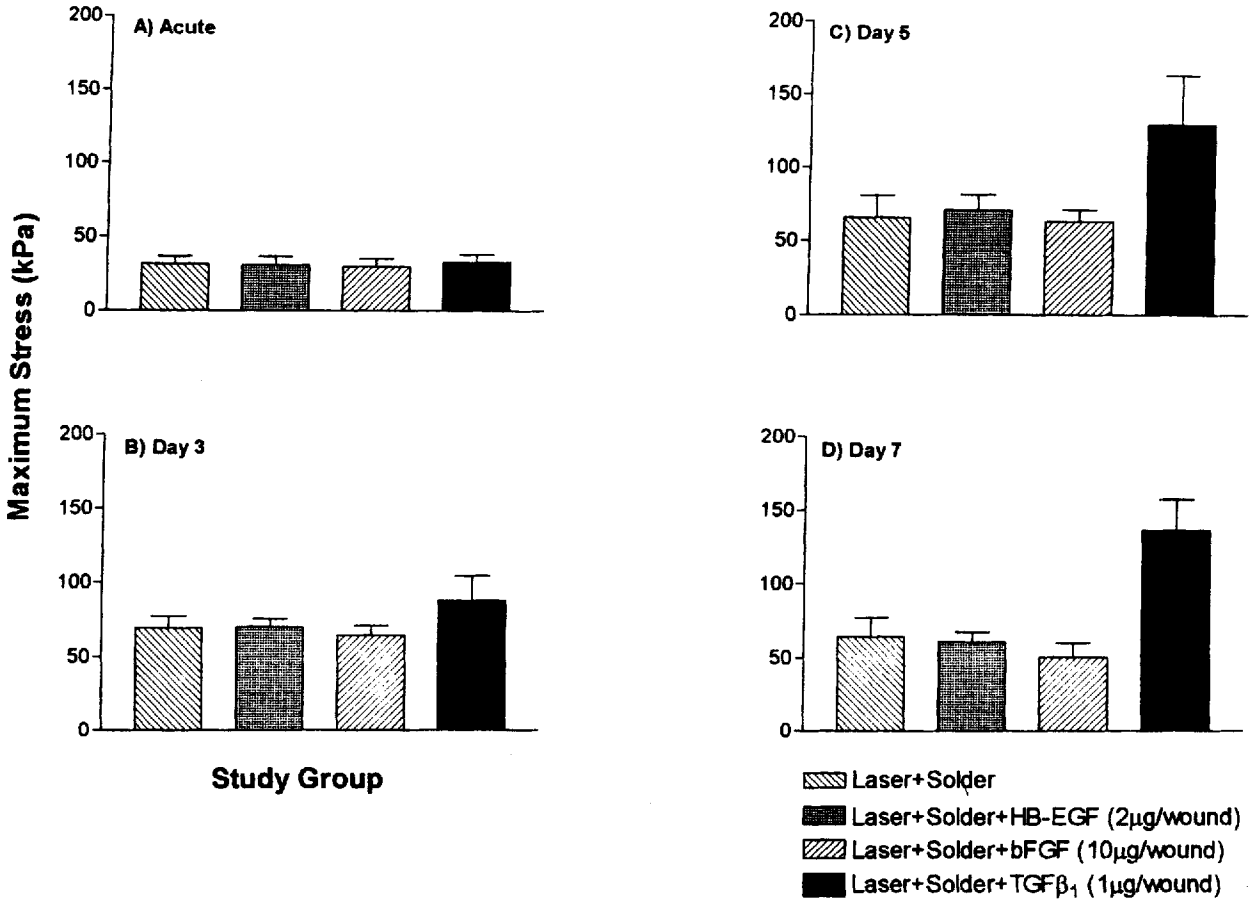

Fig. 3. Measurements of tensile strength (kiloPascals) following (A) immediate repair; (B) Day 3; (C) Day 5; and (D) Day 7 following surgery. The TGF-β_1 group at 5 and 7 days demonstrates a significant increase in tensile strength when compared to all other groups ($P < 0.05$).

LATR closures using albumin + TGF-β_1 solders enhanced the wound healing process (compared to conventional wound closure by sutures and LATR closures using either a pure albumin solder or the albumin solders prepared with two other growth factors).

DISCUSSION

This report describes two novel findings. First, we have demonstrated the feasibility of using human albumin protein solder as a carrier for the delivery of a biologically active recombinant growth factor for laser wound repair. Second, we have demonstrated that TGF-β_1, delivered in this manner to incisional wounds, can accelerate healing (compared with conventional suture closure and with photothermal wound closure using a protein solder alone). This was verified by functional measurements of wound strength, and by histologic evaluation of healing at the wound site. This effect on healing is likely to reflect both the unique effects of TGF-β_1 on extracellular matrix accumulation and its selective retention by the human albumin solder (see Fig. 2), since several other growth factors at similar concentrations, did not affect the rate of healing in this surgical model. This is the first demonstration that the incorporation of a biological response modifier (growth factor) into a protein solder used in laser-mediated tissue repair significantly accelerates wound healing.

Growth factors are soluble polypeptides that mediate multiple cellular events, including pro-

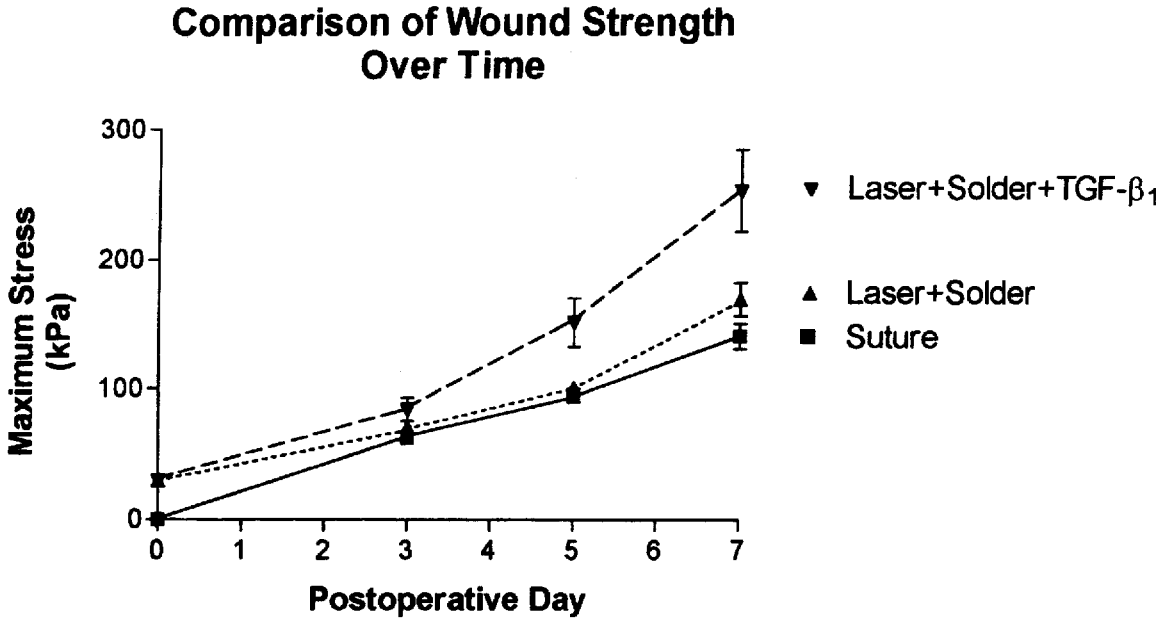

Fig. 4. Comparison of wound strength over time indicates that both laser groups have higher initial tensile strengths when compared to suture repairs ($P < 0.05$). The TGF-β_1 group achieves a strength at 4 days that is equivalent to the strength of the suture repair at 7 days. This denotes a 3 day acceleration in wound healing.

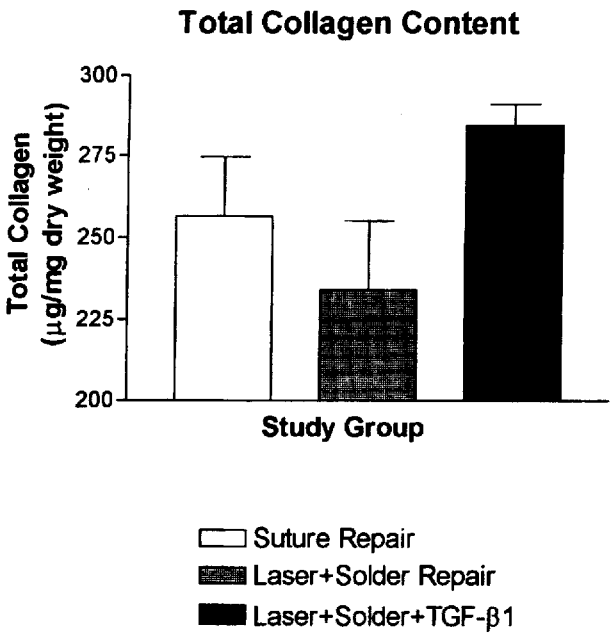

Fig. 5. Hydroxyproline assay of wounds at 7 days illustrates a higher total collagen content in the TGF-β_1 group. This indicates that an increase in total collagen may be partially responsible for the greater overall strength of the TGF-β_1 wounds.

liferation and differentiation, by binding to and activating high affinity surface receptors on target cells. Induction of growth factor gene expression, protein synthesis, and release from cells into the extracellular milieu occur during wound healing [13,19]. In addition, specific growth factors are potent mitogens for discrete classes of cells known to migrate into healing wounds, such as keratinocytes, fibroblasts, endothelial cells, and monocyte-derived macrophages. Through specific activation of subsets of differentiated cells, growth factors also mediate collagen deposition, matrix remodeling, cell turnover and angiogenesis. Thus it has become widely accepted that growth factors are crucial mediators of the wound healing cascade.

Attempts have been made to use recombinant growth factors to facilitate and accelerate would healing [13,14,19]. A number of reports have indicated that specific growth factors applied exogenously to acutely excised wounds are bioactive and can accelerate healing in model systems [26–28]. To date, there is little consensus on which of a large number of candidate growth factors are likely to be clinically useful. This is due, in part, to inconsistencies in the outcomes reported for growth factors tested in a variety of experimental models. These results may reflect significant variability in growth factor delivery, including different dosages and application protocols in the model systems used to date. Ultimately, improvements in growth factor delivery technology and in meth-

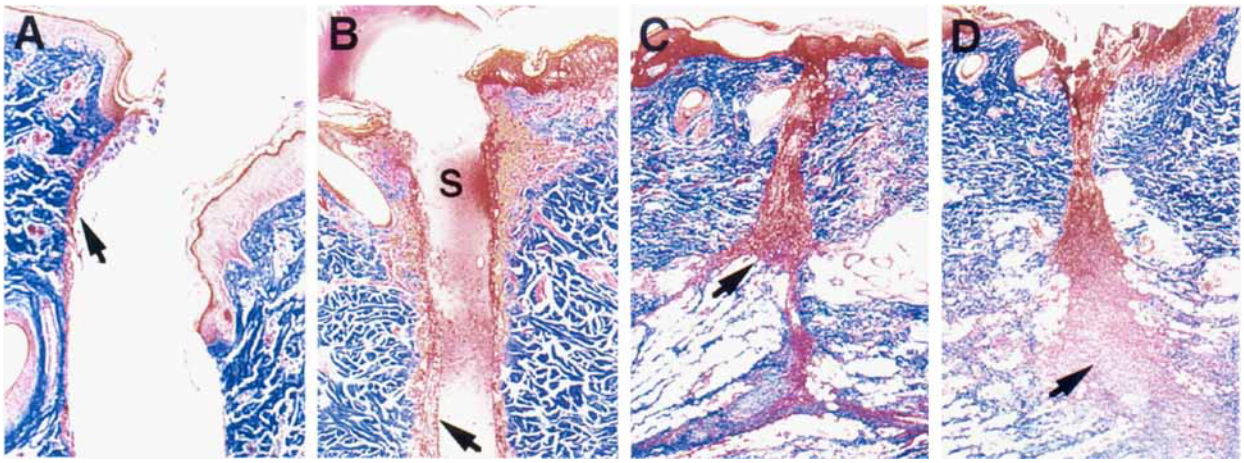

Fig. 6. Histology of laser welded wounds stained with Masson's trichrome reveals: (**A**) Normal wound edge (arrow) immediately following incision (60×), (**B**) Laser welding wound immediately following repair demonstrating superficial thermal changes along wound edge (arrow), and a column of denatured solder filling the gap between wound edges (S) (40×), (**C**) Laser welded wound on post-operative day 7 new collagen deposition at wound base (arrow) (10×), (**D**) Laser welded wound incorporating TGF-β_1 illustrating a marked increase in new collagen deposition at 7 days (arrow) (10×).

ods of evaluating potential in vivo effects may be necessary to evaluate candidate biological modifiers in diverse clinical settings.

The use of human albumin as a vehicle for the delivery of bioactive substances has several advantages; (1) Human serum albumin is known to bind reversibly to both cationic and anionic molecules. This binding affinity makes its location in plasma optimal for the transport of a number of substances, including trace metals, drugs, dyes, fatty acids, hormones, and enzymes [29]. This property may also play a role in its ability to act as a vehicle for the delivery of biologically active substances into wounds. In this regard, microspheres made from albumin have been tested experimentally as a drug delivery vehicle [30]. (2) Human albumin solders are non-immunogenic, and can be produced inexpensively in a sterile, viral-free form [29,31]. These features should enhance their use as a medical tool. In fact, several clinical applications have been developed for human albumin, which include intravenous volume expansion, and as a coating for vascular grafts, with no documented reports of viral transmission or adverse immunologic sequelae [31].

The wound closure methodology described here has several advantages as a surgical model for delivery of growth factors important in wound healing. First, the use of the temperature controlled LATR technique, in which a constant predetermined tissue surface temperature is maintained during wound closure, affords a higher degree of reproducibility in the laser assisted closure, and can also be used to prevent significant thermal degradation of the growth factors. The use of an albumin carrier, which is denatured during the LATR process, may allow the delivery of the bioactive substance in a single dose, which can be held at the wound site for extended periods of time. In this regard, remnants of the denatured albumin solder are observed 7 days after LATR closures (Fig. 6). Finally, the skin incisional model used here is an established system to evaluate growth factor mediated wound healing since porcine skin is morphologically similar to human skin. For these reasons, the novel surgical model we describe may be useful for the evaluation, in a clinically relevant experimental format, of a variety of bioactive molecules as potential mediators of healing.

Laser tissue welding using human albumin solder has been demonstrated to afford improvements over current methods of sutured wound closure [2,5,6,32,33]. By using human albumin solder as a vehicle for the site specific delivery of biological modifiers such as growth factors, we can add significantly to the future clinical applications of laser wound repair.

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